278), pore region (279-354), and post-pore region including C-terminus (>354). Cardiac events observed during follow-up from birth till age of last contact or age 40 years were defined as syncope, cardiac arrest, or sudden death.
Results. There were 117 (54%) LQT1 patients with pre-pore mutations, 84 (39%) patients with pore mutations, and 15 (7%) patients with post-pore mutation. Table shows a comparison of clinical, ECG and cardiac events data between three groups: no significant differences were observed between groups.
Conclusions. There are no significant differences in clinical presentation, ECG parameters, and cardiac events among LQT1 patients with different location of KCNQ1 mutation. These findings indicate that other modulation factors than location of mutation influence variable penetrance of the gene in LQT1 patients.

LQT1 Patients by Mutation Location

	Pre-Pore (n=117)	Pore (n=84)	Post-Pore (n=15)
Females	56 (48%)	49 (58%)	9 (60%)
Mean QTc (ms)	491	489	508
Cardiac Events	57 (50%)	33 (39%)	4 (31%)
Mean age at 1st cardiac event (years)	11	12	12

1132-125 Connexin 43 Expression Is Elevated in Human Chronic Atrial Fibrillation Related to Coronary Heart Disease

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Background: Atrial fibrillation (AF), a cardiac arrhythmia arising from multiple atrial re-entrant circuits, has a tendency to become chronic and persistent with time. Such chronicity of AF is associated with electrophysiological remodeling of the myocardium. As gap junctions are responsible for cell-cell electrical coupling, the present study assessed remodeling of the three main cardiac myocyte gap junctional proteins, connexins 43, 40 and 45, in patients with chronic AF (> six months) and underlying coronary heart disease. **Methods**: Right atrial appendage samples were collected from ten patients undergoing coronary by-pass surgery. Pre-operatively six of the patients were in sinus rhythm (SR) and four in chronic AF. Samples were immediately frozen in liquid nitrogen for analysis by western blotting and immunocytochemistry. Quantitative western blotting was undertaken for connexins 43 and 40. Immunolabeled connexins 43, 40 and 45 were examined by confocal microscopy. **Results**: Connexin43 protein levels were significantly elevated ($p<0.03$) in patients with chronic AF compared to those in SR, whereas connexin40 protein levels did not differ between the two groups ($p=0.62$). In patients with SR, the immunolabeled gap junctions were seen predominantly at the intercalated discs of atrial myocytes, with only little labeling at the lateral membranes; in patients with AF, the gap junctions appeared to be distributed more evenly throughout the cell membranes. **Conclusions**: We have demonstrated that in patients with underlying coronary heart disease, the level of connexin43 in chronic AF is elevated compared to that in SR; connexin40 levels remain unaltered. This connexin43 remodeling may contribute to the resistance of converting AF to SR with time. Although altered connexin43 expression has previously been implicated in human ventricular arrhythmias, this study suggests a wider involvement of this connexin to include arrhythmias originating in the atria. The present data do not conform to those reported from some animal models of AF, emphasizing the need for caution in extrapolating from these models to the human setting.

1132-126 Phospholemman Modulates Sodium-Calcium Exchange in Adult Rat Myocytes

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Background: The physiological role of phospholemman (PLM) is largely unknown except that PLM phosphorylation in response to adrenergic stimulation parallels a positive inotropic effect and that expression of PLM is increased 2-fold in post-infarction rat myocytes. We previously demonstrated that PLM overexpression in adult rat myocytes results in altered contractility and cytosolic calcium concentration transients. This change in contractility mimics that seen in postinfarction rat myocytes, where the sodium-calcium exchanger (NCX1) activity has been shown to be depressed. We tested the hypothesis that PLM effects on myocardial contractility is due to modulation of NCX1 function. **Methods:** Cardiac myocytes were isolated from the left ventricular free wall and septum of male Sprague-Dawley rats. Recombinant replication-deficient adenovirus (Adv) expressing either green fluorescent protein (GFP) alone, or GFP and PLM, or GFP and NCX1 were constructed and used to infect myocytes. After 72 hours, myocyte contraction was measured in medium containing 0.6 mM and 5 mM calcium. Caffeine induced contracture were performed to evaluate forward NCX1 function. Whole cell patch clamp recordings were performed to measure reverse sodium-calcium exchange current and action potential. **Results:** Half time of relaxation from caffeine-induced contracture, an estimate of forward NCX1 activity, was prolonged 1.8 fold in myocytes expressing PLM when compared to control myocytes expressing GFP alone. Reverse NCX1 current was significantly lower in PLM myocytes, particularly at more positive voltages. At 5 mM extracellular calcium the depressed contraction amplitude in PLM myocytes were increased towards normal by co-expression of NCX1. At 0.6 mM extracellular calcium the supranormal contraction amplitudes in PLM infected cardiac myocytes were reduced by NCX1 co-expression. Immunofluorescence demonstrated co-localization of PLM and NCX1 to the sarcolemma and T-tubules. **Conclusions:** PLM modulates cardiac myocyte contractility in part by altering NCX1 function. We also speculate that overexpression of PLM may play a role in the contractile abnormalities observed in post infarction cardiac myocytes.

1132-127 A DNA Microarray Survey for Gene Expression Changes in Atrial Fibrillation

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Background: Atrial fibrillation (AF) is believed to arise from very small re-entrant circuits but the underlying electrophysiological and molecular mechanisms remain unclear. We sought to identify a set of genes that are differentially expressed in AF.
Methods: Discarded atrial appendage tissue was obtained from 6 patients with AF (AF1-6) and 6 normal sinus rhythm patients (NS1-6), all undergoing coronary artery bypass graft surgery. Four pools were made; NSP1 (NS1-3), NSP2 (NS4-6), AFP1 (AF1-3), and AFP2 (AF4-6). RNA was extracted and analyzed using Affymetrix U95 subA GeneChips containing 12,625 genes. Samples were analyzed individually (AF1-6) or as "pools" where equal amounts of RNA were mixed prior to labeling and hybridization on the GeneChip. This experimental pooling technique allowed us to minimize inter-individual variation yet maximize differences that appeared solely as a function of AF.
Results: A total of 6,893 genes had detectable expression. HERG mRNA was down regulated by 33% in AF patients ($p<0.05$), whereas there was an up regulation in mRNA of ADAM 15 (37%), Calpain 1 (18%), Troponin C (30%), and Integrin α1 (79%) ($p\leq0.05$).
Conclusions: Our preliminary findings indicate that AF is accompanied by alterations in mRNA levels of several genes, including those that code for ion channels (HERG), proteins involved in atrial structure (Troponin C and Integrin α1), and mediators of inflammation or Ca^{2+} induced atrial remodeling (ADAM 15 and Calpain 1). This research may ultimately lead to identification of potential diagnostic and therapeutic targets for AF.

1132-128 Alpha$_{1a}$-Adrenergic Receptor Polymorphism Influences Baroreflex Sensitivity and Nonsustained Ventricular Tachycardia in Idiopathic Dilated Cardiomyopathy

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Altered baroreflex sensitivity (BRS) has previously shown to identify patients (pts) with chronic heart failure (CHF) at risk for non-sustained ventricular tachycardia (NSVT). Since α- and β-adrenergic receptor (AR) are involved in either the pathophysiology of CHF and in the autonomic control of heart rate, we evaluated whether α- and β-AR polymorphic variants are associated with NSVT and/or lower spontaneous BRS.
One hundred and fifty-two unrelated pts (age 49±14 years, 116 males) with DCM (WHO criteria), while receiving optimal treatment, underwent a 24-hour ECG recording, and were genotyped for the Ser49Gly and Arg389Gly polymorphisms of the β1-AR, the 5' leader cistron Arg19Cys, Arg16Gly, Gln27Glu, and Thr164Ile polymorphisms of the β2-AR, the Trp64Arg polymorphism of the β3-AR, and the Arg492Cys polymorphism of the α1a-AR. The allelic variants were characterised on the basis of PCR amplified DNA using RFLP analysis. In 77 pts it was possible to evaluate spontaneous BRS (msec/mm Hg) by using the sequence method.
We found a significant association between the Arg492Cys polymorphism of the α1a-AR and NSVT (Table). Moreover, pts carrying homozygosity for the Arg492 allele had significantly lower BRS in comparison with those who were heterozygous and homoxygous for Cys492. No significant differences were found among the other genotypes in terms of NSVT and BRS.
In conclusion, our data show that homozygous genotype for Arg492 is associated with depressed BRS and a higher occurrence of NSVT in DCM.

	HomoArg	Hetero	Homo Cys	p
Number	34	79	39	
NYHA class	1.8±0.8	1.8±0.7	1.9±0.6	NS
Ejection Fraction (%)	38±11	37±10	36±12	NS
NSVT (%)	50	23	36	0.015
BRS (msec/mm Hg)	6.5±2.7	11.9±9.0	15.4±9.7	0.022

1132-129 Delayed Ischemic Preconditioning Enhances Bcl-2 Expression and Regulates the Mitochondrial Permeability Transition Pore

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Background : The second window of protection (SWOP), beginning from 24 hours and lasting up to 72 hours following the brief episodes of coronary artery occlusions has been well demonstrated, but the exact mechanism of this protection still remains controversial. Here we have demonstrated the role of mitochondrial permeability transition pore, a non-specific pore in the inner membrane of the mitochondria, in mediating this protection. Methods and Results :Ischemic Preconditioning (IP) was induced in rats by four 3 minutes left coronary artery occlusions, each separated by 10 minutes of reperfusion on day 1. 30 minutes of ischemia and 120 minutes of reperfusion on day 2 revealed, less area of myocardial infarction (MI) in the IP group (15.32% to 45.6%, SWOP vs. sham), well maintained cardiac functions (heart rate, rate pressure product and dp/dt), well maintained myocardial ATP ($P < 0.03$), less tissue water content (73.2% to 90.6%, SWOP vs. sham), increased expression of Bcl-2, prevention of mitochondrial swelling and cyto-

chrome C release and inhibition of pathological apoptosis (2.6% to 12.4%, SWOP vs. sham) during the late phase of ischemic preconditioning. Opening the permeability transition pore before the sustained ischemia on day 2 with the PTP activator Ionidamine (10 mg/kg body weight) completely abolished these cyto protective effects of SWOP. Conclusion: The present study thus suggest the potential role of PTP in mediating the protection during second window of ischemic preconditioning by up regulating the Bcl-2 expression.

1132-130 C(-260)→T Polymorphism in the Promoter of CD14 Receptor Gene Is Associated With the Risk of Acute Coronary Events in Patients With Angina Pectoris

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Background: Inflammation and infection have been suggested to play a role in atherosclerosis and its complications. The CD14 receptor is a mediator for the activation of monocytes by lipopolysaccharide of gram-negative bacteria. The T allele of the C(-260)→T polymorphism in the promoter of CD14 receptor gene has been shown to enhance transcriptional activity and expresion of CD14 receptor on monocytes. The aim of this study was to assess whether this polymorphism is associated with increased risk of developing acute coronary syndromes (ACS) and atherosclerosis.

Methods: We studied 428 patients who underwent heart catheterization between 1994 and 1997. Stenoses ≥ 50% in at least one coronary artery were seen in 334 patients, whereas 94 had a normal angiogram. Patients with coronary artery disease were subdivided in two groups: 1) no history of ACS (n: 140; 64±9 years; men: 79%) and 2) patients with a history of ACS (n: 194; 64±9 years; men: 80%). CD14 genotypes were determined by a Polymerase Chain Reaction technique (PCR-RFLP). Genotype frequencies between different groups were compared by the χ^2-test and logistic regression adjusted by age, body mass index and conventional cardiovascular risk factors.

Results: Genotypes were in Hardy-Weinberg equilibrium. Patients with prior ACS had a significantly higher frequency of the T/T genotype than patients without a history of ACS (33% vs. 20.0%; P=0.009). After adjustment, genotype T/T was found to be an independent risk factor for ACS (OR 1.84 [1.1 to 3.1] CI 95%; P=0.023). When normal patients and patients with prior ACS were compared, the OR after adjustment was 3.1 [1.3 to 7.4] CI 95% (P=0.012). T/T genotype was not significantly different between patients without a history of ACS and normal patients (20.0% vs. 22.3%; P=0.67).

Conclusions: C(-260)→T polymorphism in the promoter of the CD14 gene is associated with a history of ACS, and it may represent a genetically determined risk factor for ACS. The response of monocytes to infectious stimuli determined by this polymorphism could play an important role in atheromatous plaque vulnerability.

1132-131 Extrinsic Rapid Electrical Stimulation Modulates Sarcoplasmic Reticulum Ca^{2+} Regulatory Proteins in Cultured Rat Cardiomyocytes

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Background: Tachycardia is commonly present in patients with heart failure (HF). However, its role in the development of failing myocardium has received little attention. We studied the effects of rapid electrical stimulation (RES) of contraction on sarcoplasmic reticulum (SR) Ca^{2+} regulatory proteins in cardiomyocytes in vitro. Methods: Neonatal rat ventricular myocytes were cultured as confluent monolayers and then subjected to RES (3.0Hz) for up to 180 min. The expression of SR Ca^{2+}-ATPase (SERCA), ryanodine receptor (RyR) and inositol 1,4,5-trisphosphate receptor type 1 (IP3R) were identified by Western blot method and RT-PCR. Contraction and relaxation characteristics of cardiomyocytes were monitored with contractility data acquisition systems. Results: The expression of SERCA protein significantly increased after 60 min compared with control (0.50±0.17 vs 0.24±0.08, p<.05), and its level returned to the control level after 180 min stimulation. The expression of SERCA mRNA tended to decrease after 180 min of RES, though not significantly, compared with control. The expression of RyR protein significantly increased after 180 min (1.76±0.56 vs 0.68±0.45, p<.05), however RES did not affect the expression of RyR mRNA. RES also caused a significant increase in IP3R protein expression, however its mRNA level significantly decreased after 180 min (0.034±0.017 vs 0.084±0.049, p<.05). There were no significant differences in terms of the time to peak tension, the time to 70% tension-regression, departure velocity, and return velocity. Conclusion: In the early stage of RES, in vitro, the expression of SR Ca^{2+} regulatory proteins was compensated due to decreased protein degradation in a different time course, leading to the maintenance of contraction-relaxation characteristics.

1132-132 Glycoprotein 130 Mediated Induction of Vascular Endothelial Growth Factor in Human Adult Cardiac Myocytes

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Background: Vascular endothelial growth factor (VEGF-A) is an endothelium-specific growthfactor. It induces proliferation, migration and NO-synthesis in endothelial cells and is able to stimulate neoangiogenesis in ischemic organs. A significant increase of VEGF-A serum levels was shown after myocardial infarction. These data suggest the importance of the VEGF system during reperaration and neovascularization. Recent data showed that glycoprotein 130 (gp130) is involved in the regulation of VEGF-A. Therefore we investigated whether oncostatin-m (OSM) or Leukemia Inhibitory Factor (LIF) are possible regulators of VEGF-A in Human adult cardiac myocytes (HACM) in vitro and thus might contribute to the neoangiogenesis during cardiac repair processes.

Methods: HACM were isolated from recipients' hearts after heart transplantation and characterized by positive staining for actin, troponin-I and cardiotin. The cells were negative for two fibroblast-specific antibodies as well as for desmin and vWF indicating the absence of fibroblasts, smooth muscle cells and endothelial cells. Such characterized HACM were treated with OSM or LIF for 24 hours and VEGF-A was determined by a specific ELISA in the conditioned media of these cells. We performed a RT-PCR in order to detect gp130, Interleukin-6-receptor (IL-6R), LIF-receptor (LIFR) or OSM-receptor (OSMR).

Results: We showed that OSM, but not LIF increased VEGF-A expression in HACM dose-dependently. The effect of OSM could be reversed using AG490, a specific JAK Inhibitor, indicating that OSM increases VEGF-A expression via the JAK/STAT pathway. These results could be confirmed on the level of specific mRNA expression as determined by RT-PCR. We detected the expression of gp130 and OSMR and to a lesser extent LIFR and IL-6-R on HACM by RT-PCR.

Conclusion: Our data suggest, that selective expression of the il-6 superfamiliy-receptors on cardiac myocytes might be involved in the induction of VEGF-A mediated neoangiogenesis in the heart.

1132-133 Trace Element Analysis of Hair Samples in Coronary Artery Disease Add Diagnostic Yield at a Bargain

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INTRODUCTION Trace elements (TE) have been implicated in the pathogenesis of diseases including cancer (Se), diabetes (Ch), and CAD (Fe). The link is causal in some: Keshan disease (Se), and associative in others; Down's syndrome (Ca). Further, specific interactions are not well understood, in part related to complexities of traditional harvesting, preparation and analysis of low concentration samples. Clearly, an improved method would be advantageous. Since hair samples offer an atraumatic technique with a ten-fold higher concentration of TE than serum, we investigated the feasibility of TE analysis in hair of pts with documented (CAD).

HYPOTHESIS We hypothesized that using a new, highly sensitive technique, Inductively Coupled Plasma Mass-Spectrophotometer (ICP), TE may offer a novel non-invasive and inexpensive manner in which to screen populations for CAD.

METHODS Above root hair was obtained in 20 subjects; 10 pts with simultaneous routine cardiac catheterization and lesions >60%(CAD+). Ten subjects without clinical evidence of CAD served as controls(CAD-). Analysis was conducted on a double focusing sector field resolution ICP with a linear dynamic range greater than 100. Multi-element screening of 16 TE's (Cd, Co, Cr, Cs, Cu, Fe, Mg, Mn, Pb, Rb, Sb, Se, Sn, Sr, Tl, and Zn) with ultra-high resolution and low dark noise resulted in detection of TE to pcg/L. Calibrated preparation: acetone, double distilled H_2O, and digestion with 20% high purity NHO_3 at 60°C for 2 weeks prior to analysis. Mann-Whitney U and Newman-Keuls were used to test for significance.

RESULTS No significant relationship of TE with HTN, diabetes, race or family history was found. However, TE concentrations diverged between the CAD+ and the CAD- group in 14 of 16 TE's. Mean TE concentrations in the CAD+ patients were lower than CAD- patients in Cd, Co, Cr, Cs, Cu, Fe, Mg, Mn, Pb, Rb, Sb, Se, Sn, and Zn and higher for Sr and Tl ($p<0.05$). Analysis cost: $9.37/ hair sample.

CONCLUSION Trace element hair sample analysis by Inductively Coupled Plasma-Mass Spectrophotometer with a standardized preparation offers the potential for additive noninvasive, inexpensive and sensitive information in the evaluation of patients with suspected CAD.

POSTER SESSION

1133 Predictors of Risk in Hypertensive Patients

Monday, March 31, 2003, 3:00 p.m.-5:00 p.m.
McCormick Place, Hall A
Presentation Hour: 4:00 p.m.-5:00 p.m.

1133-117 Repeated Exposure to Caffeine Increases Arterial Stiffness

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Background: Caffeine (C) is the most widely consumed pharmacological substance. Wave reflection (WR) along the arterial tree, an important index of arterial stiffening and cardiac afterload, is involved in the pathogenesis of hypertension. We studied the effect of C on WR and especially that of repeated exposure because of the possible tolerance that develops to C.

Methods: Twelve healthy volunteers (age 29±4 yrs) were studied in a randomized, placebo-controlled, crossover fashion (100 mg of C orally -equivalent to 1 cup of coffee- and 120 min later another 100 mg of caffeine). WR was evaluated using a validated system (Sphygmocor®) that employs (i) high-fidelity arterial tonometry for the non-invasive registration of arterial pulse and (ii) appropriate computer software for pulse wave analysis. Augmentation index (AIx) was measured as an index of WR.

Results: The first dose of C led to a substantial increase in AIx indicating increased effect of WR from the periphery. The second dose increased AIx again, but to a lesser extent (figure). Aortic pressures also increased (systolic: by 2.6 with the 1st dose and by 2.7 mmHg with the 2nd; diastolic: by 4.3 and by 1.3 mmHg respectively).